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Research Report

The distribution of *Streptococcus mutans* and *Streptococcus sobrinus* in children with dental caries severity level

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ABSTRACT

Background: *The prevalence of dental caries is high worldwide and specifically in Indonesia, especially in children. Cariogenic bacteria are the major cause of dental caries. Streptococcus mutans (S. mutans) is one of the bacteria often associated with caries, due to its ability in producing acid and forming the biofilm for bacterial colonisation on the surface of oral cavities. In addition to S. mutans, Streptococcus sobrinus (S. sobrinus) bacteria are also thought to play an important role in the process of caries.* ***Purpose:*** *This study aims to analyse the distribution of S. mutans and S. sobrinus in children with seriously high dental caries levels.* ***Methods:*** *This study was an observational analytical study. Bacterial isolation was conducted in carious lesions of 50 paediatric patients 6-12 years old with superficial dental caries. Samples of caries lesions were put directly into a tube containing the Brain Heart Infusion Broth (BHI-B) and incubated at 37^{o} C for 24 hours. The samples were sub-cultured on selective tryptone yeast cystine sucrose bacitracin (TYCSB-Himedia) agar, and then incubated for two days. Bacterial identification was then performed using the polymerase chain reaction (PCR) Multiplex method. Statistical analysis with Chi-square.* ***Results:*** *The total number of children with dental caries included in this study was 50. Among these, 94% showed positive for S. mutans and 30% positive for S. sobrinus. The analysis of the prevalence of bacterial colonisation (S. mutans and S. sobrinus) based on caries severity and the Simplified Oral Hygiene Index (OHI-S), showed there was no significant difference (p> 0.05).* ***Conclusion:*** *This study showed that among 50 caries noted in the children, 94% were colonised S. mutans and 30% S. sobrinus. There was no significant difference between the colonisation of S. mutans and S. sobrinus among children from the severe to mild decayed exfoliated filling teeth (DEFT) category, and between bad and good OHI-S.*

Keywords: *caries severity; dental caries; OHI-S; Streptococcus mutans; Streptococcus sobrinus*

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INTRODUCTION

Dental caries is a serious oral health problem in Indonesia and the rest of the world. Based on the 2018 Basic Health Research (RISKESDAS) data, the prevalence of caries in Indonesia was significantly high, above the World Health Organization (WHO) target.[1,2] According to the RISKESDAS data, the prevalence of caries reached 93% in children aged between five and six years, while WHO and Federation Dentaire Internationale (FDI) had a target to make 50% of children free of dental caries. The decay missing filling teeth (DMFT) index for primary teeth in children at these ages was 8.43, indicating severe early childhood caries were found in roughly nine teeth per child.

Moreover, dental caries is mostly caused by cariogenic bacterial infections. *Streptococcus mutans* (*S. mutans)* is the main cariogenic bacterium in the pathogenesis process of caries.[3] *Streptococcus sobrinus* (*S. sobrinus*) is also thought to play a role in the production of caries.[4] The pathogenesis process for dental caries involving *S. mutans* usually starts with bacterial colonisation. *S. mutans* biofilm then produces

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organic acids as a by-product of fermentable carbohydrate metabolism. This acid can cause the local pH to fall below the critical value, resulting in the demineralisation of dental tissue. One of the results of *S. mutans* producing high cariogenicity levels is its ability to adhere to the surface of a tooth. This attachment is successfully performed by extracellular polysaccharides (EPS) derived from sucrose. This process also involves the microbiological characteristics of the bacterial cell wall structure.[5]

In most cases, *S. mutans* is the main cause of caries. Nevertheless, the role of acidogenic and other aciduric bacteria, such as *S. sobrinus,* is also assumed to be important. Based on several epidemiological and in vitro studies, *S. sobrinus* can be more cariogenic than *S. mutans.*[6] The virulence of the *S. mutans* group is related to its ability to colonise and develop on the tooth surface during acidic conditions. These properties include the production and regulation of adhesion proteins, glucosyltransferases (GTF), and extracellular polysaccharides, such as glucans which allow bacteria to attach firmly to the surface of teeth in biofilms. The two species (*S. sobrinus* and *S. mutans*), however, have different strategies in their attachment mechanism. *S. mutans* uses pellicles and specific surface antigens directly, while *S. sobrinus* uses glucans.[7]

Previous research in Mongolia also showed that children aged between five and seven with both *S. mutans* and *S. sobrinus* in their saliva had significantly more dental caries than those who had only *S. mutans* or *S. sobrinus.*[8] In test animals, *S. sobrinus* can produce more acids than other species in the *S. mutans* group. The prevalence and level of *S. mutans* and *S. sobrinus* colonies, as a result, have been used as biological markers for caries prediction.[9] This study aims to analyse the distribution of *S. mutans* and *S. sobrinus* in children with severe levels of dental caries.

MATERIALS AND METHODS

This study was approved by the ethics committee number: 328/HRECC.FODM/VI/2019 of the Faculty of Dental Medicine, Universitas Airlangga. This study was an observational analysis research. Samples were collected October 2nd-10th, 2019. The Dental and Oral Hospital, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, supplied samples from 50 paediatric patients. The patients were aged 6-12 years. Parents of these paediatric patients signed informed consent. The teeth examined were deciduous molars. The types of caries examined were superficial caries or caries media. Next, the severity of the caries was analysed based on decayed exfoliated filling teeth (DEFT) and the Simplified Oral Hygiene Index (OHI-S). *S. mutans* and *S. sobrinus* were then identified with multiplex polymerase chain reaction (PCR).

The caries lesions were taken from the first molar teeth using a sterile excavator and placed directly into the bottom of a tube containing Brain Heart Infusion Broth (BHI-B) (Merck, Darmstadt, Germany). Next, the tube was put into an incubator at 37°C for 24 hours. On the second day, the lesion was sub-cultured on selective Tryptone Yeast Cystine Sucrose Bacitracin (TYCSB) (Himedia, Himedia Laboratories Pvt Ltd, India), and then incubated for two days. The growth of the colonies was indicated by the macroscopic characteristics of the colony of *S. mutans,* such as the hardened and sticky crystal form on the media, which were then examined with PCR.

The PCR multiplex method was used to detect *S. mutans* and *S. sobrinus* bacteria. The results of the amplification were then visualised using an electrophoresis method. DNA extraction was performed using the boiling method in TE buffer. A suspected three to five colonies on TYCSB media were taken and inoculated in Eppendorf tubes containing 100 µL of TE buffer, and homogenised by vortex mixer. The suspension was heated at a thermostat temperature of 95°C for ten minutes (Eppendorf, North America). After the samples reached room temperature, they were centrifuged at 10,000 rpm for ten minutes. The extracted DNA in the supernatant was stored at -20°C before use as the DNA template for PCR.[10]

PCR was run 25 µL PRC mixture, as follows: 12.5 µL of dNTPmix (Dream Taq Green, Thermo Scientific, USA), 0.5 µL (50pmol), 3.5 µL of bacterial DNA template, 1Ul tag pol, and then adding 17 µL of distilled water. The primers sequence of *S. mutans* used GTF-B F :ACT ACA CTT TGC GGT GGC TTGG as forward and GTF-B R :CAG TAT AAG CGC CAG TTT CACT as reverse in 517 bp.[11] Primers sequence of *S. sobrinus* used GTF-I F : GAT AAC TAC CTG ACA GCT GAC T as forward and GTF-I R : AAG CTG CCT TAA GGT AAT CAC T as reverse in 712 bp.[12] DNA amplification was then performed using a thermal cycler PCR machine (Icyler, Biorad Thermal Cycler).[13]

The PCR was first run using a hot initial temperature of 95°C for one minute and amplified for 35 cycles with denaturations at 94°C for 30 seconds, annealing at 53°C for one minute, elongation at 72°C for two minutes, and ending at 72°C for seven minutes. PCR results were visualised using electrophoresis in 2% agarose gel (Spectronics Corporation, USA), with 100 bp marker ladder. Electrophoresis was run at 100 volts for 30 minutes. Next, the agarose gel was stained with Ethidium bromide solution for 20 minutes. The amplicons were visualised using GelDoc (Digibox 7000, Mbiotech, Korea). Positive results were shown by the presenting of amplicon 517 bp for *S. mutans* and 712 bp for *S. sobrinus*. The results were studied using descriptive analysis of the distribution of *S. mutans* and *S. sobrinus* with caries severity, and then statistically analysed with the Chi-square test.

RESULTS

After conducting research, bacterial isolates obtained in this study were identified by multiplex PCR to confirm *S. mutans* and *S. sobrinus* (Figure 1). Figure 1 showed results of multiplex PCR positive *S. mutans* (GTF-B) in the

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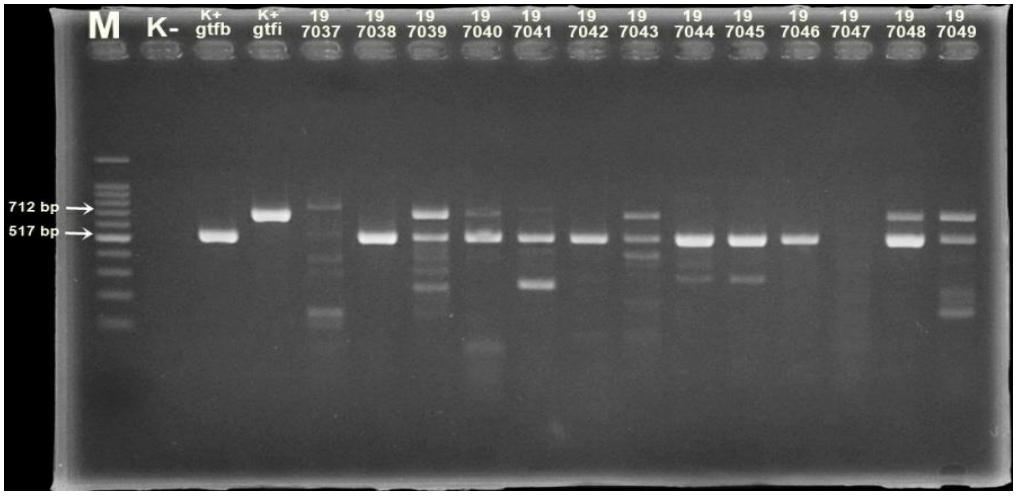

Figure 1. The results of multiplex PCR on *S. mutans* (GTF-B) and *S. sobrinus* (GTF-I).

Table 1. The isolation rate of *S. mutans* and *S. sobrinus* among children (n=50) with various dental caries severity level from patients visiting Dental and Oral Hospital Universitas Airlangga

Severity Level		Children with dental caries (n=50)				
		S. mutans (n=47=94%)	p value	*S. sobrinus* (n=15=30%)	p value	Not identified *S. mutans* and *S. sobrinus* (n=3=6%)
DEFT	Mild	12 (24%)	0.124	3 (6%)	0.409	2
	Severe	35 (70%)		12 (24%)		1
OHI-S	Good	35 (70%)	0.315	9 (18%)	0.083	3
	Bad	12 (24%)		6 (12%)		0

number 7038, 7039, 7040, 7041, 7042, 7043, 7044, 7045, 7046, 7048, 7049 and positive *S. sobrinus* (GTF-I) in the number 7039, 7043, 7048, 7049. The results were then grouped based on DEFT severity and OHI-S, then analysed by frequency distribution and Chi-square.

The prevalence of the colonisation of *S. mutans* was higher compared to *S. sobrinus,* 94% vs 30% (Table 1). Based on the level of caries using DEFT scores, severe scores for *S. mutans* were higher than mild scores. Statistically, there was no significant difference using the Chi-square test (p value >0.05). As with *S. mutans, S. sobrinus* was higher in severe scores than mild scores of DEFT, but statistically, there was no significant difference. Based on OHI-S scores, *S. mutans* good scores were higher than bad scores. Statistical tests using Chi-square showed there was no significant difference (p value >0.05). As with *S. mutans,* the good DEFT *S. sobrinus* scores were higher than the mild scores, but again, statistically, there was no significant difference.

DISCUSSION

Several studies have shown that preventive efforts are effective in deterring early *S. mutans* colonisation from causing dental caries in children.[8] Hence, this study aims to reveal the incidence pattern of *S. mutans* and *S. sobrinus* in children based on the DEFT and OHI-S. Next, the results of this study found that the highest incidence of bacteria causing dental caries was *S. mutans* (94%). Meanwhile, the incidence of *S. sobrinus* was 30%. These findings indicate the existence of *S. mutans* is considered not only as a microflora of the oral cavity but also as a pathogenic bacterium causing caries. Both *S. mutans* and *S. sobrinus* can proliferate in dental biofilm plaque. Their virulence is mainly due to their high adhesion ability, acidity, and their properties.

Moreover, dental biofilm containing cariogenic bacteria (caries-related micro-organisms) is one of the most harmful factors associated with the development of tooth decay. Dental biofilms can be found on hard surfaces in the oral cavity, such as on surfaces, implants, orthodontic devices, or restorative materials. The development of biofilm processes involves several progressive stages. The formation of initial biofilm accumulation involves specific processes. Variations in the biofilm coat in the oral cavity have a significant impact on oral ecology and dental caries development.[14]

The higher colonisation rate of *S. mutans* and *S. sobrinus,* as demonstrated in this study would be ruled

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by antigen I/II protein that strengthens the adherence to the tooth surface. It was also facilitated by glycoprotein receptors present in saliva, called salivary agglutinin.[15] The other factors are cell-to-cell adherence and development of cohesive and pathogenic biofilms via the expression of GTFs. These enzymes (140 to 160 kDa) produce extracellular adhesive glucans that vary in chain length, contain α-1,3 and α-1,6 glucosyl linkages, and have a degree of branching and solubility. *S. mutans* comprises three genes for GTF: GTF-B, responsible for insoluble glucan synthesis; GTF-C, for soluble and insoluble glucan synthesis; and GTF-D, for soluble glucan synthesis. *S. sobrinus* expresses GTF-I and GTF-S, encoding enzymes that produce insoluble and soluble glucans, respectively.[15] The prevalence of *S. mutans* and *S. sobrinus* is widely associated with caries. In several epidemiological studies, there was a correlation between the existence of *S. sobrinus* and the high incidence of dental caries.[16]

The results of this study show that there was no statistically significant difference in the incidence of *S. mutans* and *S. sobrinus* between the high and low caries severity level. This can be caused by several factors, such as host factors, bacterial virulence, diet, environment, and time. Risk factors, such as sociodemographic factors, socioeconomic factors, knowledge levels, as well as behaviour, also affect the incidence of caries.

Dental caries occurs because of an imbalance between demineralisation and remineralisation. When demineralisation is higher than remineralisation, caries can occur. Oral hygiene also has a role in this balance.[17] Nevertheless, in this study, there was no significant difference in the incidence pattern of *S. mutans*, *S. sobrinus* based on high or low DEFT and OHI-S.

Finally, this study interestingly reveals that there was no significant difference in the incidence pattern of *S. mutans* and *S. sobrinus* bacteria based on the severity of caries and OHI-S. This means that although these two bacteria are considered the main factors that cause dental caries, other factors may have an equally important role in caries. Hence, further research is expected to focus on more in-depth studies of *S. mutans* and *S. sobrinus* bacteria with other risk factors. In conclusion, this study showed that among carious teeth, 94% were colonised by *S. mutans,* and 30% of cases demonstrated co-colonisation of *S. mutans* and *S. sobrinus*. There were no significant differences in these bacterial colonisations between various levels of dental caries.

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